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E. coli in log growth have been exposed to a pulsed electromagnetic field in the presence of ^{35}S methionine. Following EMF-exposure, the cells were lysed, the proteins extracted and then analyzed by 2-dimensional polyacrylamide gel electrophoresis (2-D PAGE). In the first dimension, proteins were separated by equilibrium and non-equilibrium isoelectric focusing. The former system tends to separate the more acidic proteins while the latter gel resolves the more basic proteins (i.e. ribosomal proteins). The focused proteins are then separated in the second dimension according to their molecular weight (Mr) by an SDS-PAGE system. Of the more than 2000 proteins separated by the two systems and analyzed by computer, 13 proteins have been earmarked for identification and further scrutiny. The initial selection of these proteins was based on the requirement that the radioactive counts must differ by a factor of three. Preliminary examination of these data indicate that the newly expressed proteins are not related to the heat-shock proteins of *E. coli*. Keywords: pulsed electromagnetic fields; sinusoidal electromagnetic fields; molecule molecule interactions; (RT)

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BACKGROUND: Recently, Goodman and Henderson (1986) reported that exposing the salivary gland cells of the Dipteran, Sciara to either a pulsed or sinusoidal electromagnetic fields altered both transcriptional and translational events. To date, the identity of these newly transcribed and/or translated macromolecules have not been determined. In this laboratory, we have been subjecting Escherichia coli to a pulsed magnetic field in an attempt to elucidate the fundamental mechanism of interaction between weak electromagnetic fields and cells. → cont. in DD1473 ↩

PROTOCOL & PROGRESS: Cultures of E. coli (K-12) are maintained in the defined growth medium of Neidhardt et al. (1974). During the first year, several different experimental protocols were examined in an attempt to minimize both noise and scatter in our data. In our current protocol, a 125 ml Erlenmeyer flask containing 25 ml of growth medium is inoculated with E. coli and placed in a solenoid. The solenoids are attached to a platform mounted on a reciprocal shaker (model R-7, New Brunswick Scientific); the shaker is housed in an incubator (model RL-48, Warren Sherer) maintained at 32 °C. The cells are allowed to grow to mid-log phase; to insure that cultures are in the log phase of growth, their turbidity is compared to a set of predetermined, standard values. A 4.0 ml cell suspension is removed and re-inoculated to 21 ml of new growth medium containing 15 uCi of ^{35}S methionine, specific activity 1361 Ci/mmol (Amersham). Following the second transfer, and after an adequate cell density has been reached (about 3 hrs), two 10 ml cell suspensions are removed and placed in two sterile flasks. Each flask is then placed in one of the incubator's solenoids; one solenoid is energized to generate the appropriate pulsed magnetic field and the other solenoid serves as a non-exposed control.

Field Application: The solenoids (7.5 cm in diameter and 14.5 cm high) are wound with 80 turns of B & S #16 enamel-coated copper magnet wire on a Lucite tube. The coils are driven by 250-watt audio amplifier (Grommes-Precision model G252A), which derives its source from a Wavetek Model 275 arbitrary waveform generator. Because of the inductance of the coil, the amplifier-load combination puts a current through the load that is the integral of the input signal. Therefore, the magnetic field pulses applied to the flasks have the waveform that is the approximately the integral of the waveform generator waveform; the electric fields and currents induced in the culture medium have waveforms that are approximately the same as that of the waveform generator. The only departure from this general rule, (which in fact is a departure of which we have taken advantage) is when there is a resonant ringing of the system.

The waveform generator has been programmed to provide a variety of input signals to the system; the one used in this year's experiments has provided an approximately triangular magnetic pulse shape with the peak magnetic field calculated to be approximately 4 mT. The calculated peak electric field induced in the medium at the outer edge of the flask was about 1.7 V/m. We have been applying a burst of 22 magnetic field pulses, each of which has a rise time of about 200 usec and a fall of about 20 usec; the bursts are repeated 25 times a second.

Protein Analysis: Following a 30 minute field exposure, 1.0 ml of each cell suspension is withdrawn and centrifuged in an Eppendorf microfuge for 2 minutes. The supernatants are decanted and the pellets dissolved in 100 ul of

a lysing solution at 37 °C for two hours. The lysing solution is composed of 50 ul 0.5 M Tris-HCl buffer, pH 6.8; 80 ul 15% SDS, 20 ul glycerol 40 ul B mercaptoethanol, and 272 ul H_2O. After the pellets have dissolved, 100 ul of a second lysing buffer is added to the suspensions; the second buffer contains 0.02 M NaOH, and 2% (3-[(3-cholamidopropyl)-dimethylammonio]-2hydroxy-1-propanesulfonate [CHAPSO]). The latter lysing buffer is thought to prevent streaking in the first dimension.

Samples were isofocused to equilibrium in the first dimension and separated on an SDS-polyacrylamide gel electrophoresis (PAGE) in the second dimension. Because of the availability of a large E. coli protein data base, and the ability to quantify protein spot differences, we also had sample gels run by a commercial company (Protein Database Inc [PDI]). In these experiments, control and exposed E. coli polypeptides were isolated and separated by both equilibrium and non-equilibrium focusing system in the first dimension and then subjected to SDS-PAGE in the second dimension. Of the more than 2000 polypeptides separated by the two systems and analyzed by PDI computer, 13 proteins have been earmarked for identification and further scrutiny (see Table I.). The initial selection of these proteins was based on the arbitrary requirement that the radioactive counts in a given polypeptide must differ by a factor of three. Preliminary examination of these data indicate that the newly expressed proteins are not related to the heat-shock proteins of E. coli.

FUTURE DIRECTIONS: In the coming year, we intend to:

1. examine the effects of sinusoidal electromagnetic fields on peptide profiles and to identify those polypeptides whose appearance is altered by exposure to weak fields.

2. vary the pulse shape and peak intensity. We have been experimenting with a high-power operational amplifier-based circuit (using the Teledyne-Philbrick 1468 amplifier) that has higher frequency response and less tendency to "ring" than the audio amplifier, pushing back one limitation of the field-application chain; this limit is not as controlling as the coil inductance, however.

3. test the relative efficacy of slower and faster rising pulses on changes in protein expression within the limitations of the field-generating apparatus. The field pulses described above have strong frequency components and harmonics at both 25 Hz and at 4.8 kHz; experiments with sine waves will look at the relative effectiveness of these frequencies.

REFERENCES

Goodman, R.; and Henderson, A. (1986) Some biological effects of electromagnetic fields. Bioelectrochem. Bioenerg. 15:39-55.

Neidhardt, F.; Bloch, P. and smith, D. (1974) Culture medium for enterobacteria. J. Bacteriol. 119:736-747.

TABLE I.

Radioactivity (DPM) of proteins extracted and resolved by two-dimensional gel electrophoresis from EMF-exposed and control E. coli. Only those proteins that differ by a factor of three in their counts have been listed.

EQUILIBRIUM GEL

		EMF-exposed	Control
Mr ($x10^{-3}$)	pI	DPM	DPM
56.8	--	75	1058
59.8	4.85	392	82
47.6	4.98	307	----
43.8	4.98	319	----
139.3	5.58	80	1031
27.9	----	452	152

NON-EQUILIBRIUM GEL

		EMF-exposed	Control
Mr ($x10^{-3}$)	pI	DPM	DPM
57.8	--	998	286
43.3	--	---	1726
43.4	--	973	135
43.2	--	1303	108
42.6	--	975	64
146.3	--	---	952
110	--	---	401

[Mr = molecular weight, pI = isoelectric point, DPM = decays/min]